Effects of Anti-Epileptic Drugs on Infant Brain Networks

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Abstract

The aim of this thesis was to study effects of anti-epileptic drugs in the infant brain using functional connectivity networks defined by phase and amplitude correlations in EEG measurements. Prenatal exposure to anti-epileptic drugs is a well-known cause of problems like decreased cognitive function later in life.

Connectivity matrices derived from EEG measurements of 56 newborns with prenatal exposure to anti-epileptic drugs and 67 healthy controls were used in the analysis. EEG measurements collected during both active sleep and quiet sleep were used, and both phase-phase and amplitude-amplitude correlation was used in defining the brain networks. Network connectivity was studied using multiple network metrics that give information about network segregation and integration. In addition, correlation between network statistics and neurodevelopmental assessment in the age of two was studied.

Significant differences were found in efficiency and clustering of the networks between the AED-exposed newborns and healthy controls. Also, differences in the networks was found using a method called the network-based statistic. These results indicate that functional connectivity could be useful in the examination of the effects of anti-epileptic drugs to the infant brain.

Keywords Infant, Functional connectivity, EEG

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Tiivistelmä

Raskaudenaikainen altistuminen epilepsialääkkeille lisää lapsen riskiä muun muassa kognitiivisen suorituskyvyn alenemalle. Tämän diplomityön tavoitteena oli tutkia epilepsialääkkeiden vaikutusta vastasyntyneiden aivojen funktionaalisiin verkostoihin. Tähän käytettiin aivosähkökäyräsignaaleista sekä vaihekorrelaation että amplitudikorrelaation mukaan määriteltyjä verkostoja. Verkostoja analysoitiin graafiteoreettisin menetelmin.

Tutkimuksessa oli mukana 56 raskauden aikana epilepsialääkkeille altistunutta vastasyntynyttä sekä 67 tervettä verrokkia. Aivosähkökäyrää oli tallennettu sekä syvän että REM-unen aikana. Lasten neurologista kehitystä oli myös tutkittu kahden vuoden iässä, ja verkostoanalyysin tuloksia vertailtiin lopuksi lasten kehitykseen liittyviin tuloksiin.

Epilepsialääkkeille altistuneiden ja verrokkiryhmän aivoverkostojen välillä havaittiin eroavaisuuksia verkostojen tehokkuudessa ja klusteroitumisessa. Tulokset viittaavat siihen, että funktionaalisia verkostoja voitaisiin tulevaisuudessa käyttää apuna tutkittaessa epilepsialääkkeiden vaikutusta vastasyntyneiden aivoihin.

Avainsanat elektroenkefalografia, vastasyntynyt, verkostoanalyysi

Preface

I would like to express my gratitude to my advisors Sampsa Vanhatalo and Anton Tokariev at BABA Center, without whom I would not have had the slightest chance of ever writing a thesis. It is their expertise and kindness that made it possible for me to understand and write anything about a subject so fascinating and complex. I would also like to thank my supervisor Riku Linna for helping me through the process of finishing this work in the times I did not believe it would ever happen.

This pretty much marks the end of the best and most transformative period of my life so far. During my years as a student, I have been surrounded by some truly wonderful people, and it makes me happier than anything to recount all the unforgettable moments we have experienced together. These moments have been made possible by all my friends at Inkubio, Teekkarispeksi, Aava, ITMK, and many other great groups to which I have had the pleasure to belong. It has to be noted that anyone reading this is highly likely to have played a part in this journey. For that I am sincerely thankful, even if it is hard to find the right words to say it in person. I am looking forward to all adventures the future may hold with every one of you.

Most importantly, I want to thank Sini for being there for me. As the better half of our own small team, she has probably helped me in more ways than anyone can possibly even know.

Otaniemi, 23.9.2020

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Contents

Abbreviations

AAC	amplitude-amplitude correlation
AED	anti-epileptic drugs
AS	active sleep
BSID	Bayley Scores of Infant and Toddler Development
EEG	electroencephalography
GABA	gamma-aminobutyric acid
IHS	interhemispheric synchrony
PPC	phase-phase correlation
dWPLI	squared weighted phase lag index
SAT	spontaneous activity transient
QS	quiet sleep

1 Introduction

Anti-epileptic drugs (AEDs) are a treatment for epilepsy, a neurological condition which requires regular pharmacological management, also during pregnancy. This exposes children to a wide array of complications during pregnancy and hindrances in later life. By more efficient and early recognition of adverse effects of prenatal AED exposure, the management of neurodevelopmental disorders could be optimized beyond the current clinical practise. [1][2]

EEG is a powerful tool for evaluating the neurological state of newborns. By the use of EEG data, known for its high temporal resolution, information about the connections of the infant brain could be inferred and accurately used in analysing the neurological development of the newborn. Evaluating the interareal synchronization of elemental features of EEG, like phase and amplitude, forms the basis for functional connectivity analysis. [3]

Functional connectivity is a phenomenon increasingly studied in brain research, which deals with the temporal correlations between separate brain regions. As most neurological disorders may not present as alterations in structural connections, assessment of functional connectivity has been recognized as an essential method for finding relevant information about the brain function. For this reason, many measurements of complex network analysis have been utilized for analysis of brain networks. [4][5][6]

The dataset used in this thesis consists of brain connectivity matrices depicting both phase-phase correlation and amplitude-amplitude correlation in EEG measurements of 56 infants prenatally exposed to AEDs and 67 healthy controls. The data includes measurements from both sleep states, active sleep and quiet sleep. The EEG measurements were done at the conceptional age of 42 weeks.

The aim of this thesis was to gather information about the differences in functional connectivity between AED-exposed infants and healthy controls. For this purpose, network measures including efficiency, clustering and modularity were used. Additionally, a method called network-based statistic was used to identify differences in the networks. As neurodevelopmental assessment of the test subjects in the age of two was also available, a secondary goal was to investigate whether differences in the networks in the early life can be correlated with neurological development later in life.

2 Background

In this section, previous research around the study field of this thesis is described.

2.1 Anti-epileptic drugs

Anti-epileptic drugs (also known as anticonvulsants or anti-seizure drugs) are pharmacological substances used in the treatment of epilepsy, which is a neurological condition affecting approximately 1 percent of the general population [7].

Because of their multiple mechanisms of action, they are also used in a range of other neurological and psychiatric disorders, such as bipolar disorder and neurogenic pain [8].

Conventional AEDs are generally attributed as having an enhancing effect on GABAergic inhibitory neurotransmission or an inhibitory effect on voltage-gated sodium ion channels [9]. In addition to these mechanisms, newer AEDs can also inhibit glutamatergic neurotransmitter receptors or T-type calcium channels. [10] The mechanisms of action remain partially unclear for some AEDs currently in use.

AEDs are used in neonatal intensive care for preventing seizures that might contribute to brain injury, as they have been shown to have neuroprotective effects in such cases [11]. However, many kinds of AEDs have been shown to cause neuronal cell death when applied to a developing brain in animal studies, which indicates that the use of such drugs is not without a risk in the early stages of life [12]. This thesis focuses on infants exposed to AEDs during pregnancy leaving use of AEDs for neonatal complications outside of its scope.

2.1.1 Effects of prenatal AED exposure

Epilepsy is one of the most common neurological disorders that requires continuous treatment during pregnancy. However, the use of AEDs during pregnancy has been reported to increase the risk of both prenatal and postnatal complications. Neurodevelopmental effects of prenatal AED exposure include impaired verbal and memory abilities as shown by multiple measures of cognitive function [13][14][15]. Because of the seriousness of the neurodevelopmental outcomes, there is a need for methods for recognizing evidence pertaining to the adverse outcomes in a more time sensitive manner to be able to manage the deficits as early as possible [16]. Other adverse effects of prenatal AED use commonly include congenital malformations, fetal growth restriction and fetal death, which have been generally reported and studied more extensively than neurodevelopmental outcomes [17][1][16].

GABAergic interneurons have been offered as one of the cellular level explanations for what causes synchronization of oscillatory activity in early brain development [18]. Maturation of GABAergic brain circuits has also been shown to determine the onset of critical developmental periods early in life, and it is recognized that pharmacological targeting of these circuits can onset premature or delayed development [19].

Offspring of women with epilepsy have an increased risk of perinatal complications, and the risk is further increased by gestational use of AEDs like valproate, carbamazepine, oxcarbazepine, lamotrigine and levetiracetam [20].

It has been suggested by studies that list outcomes for individual drugs that neurocognitive deficiencies associated with AEDs are most accountable to valproate, whereas many other AEDs have no such effect [15]. For example, a higher occurrence of ADHD has been reported in children prenatally exposed to valproate, but not in children exposed to other AEDs [21]. Valproate is listed as a first-line drug for all epilepsy-related complications in the U.S. [22] and it is also listed as an essential medicine according to the World Health Organization [23].

In this thesis, it is only taken into account whether the infant has been prenatally exposed to AEDs or not, without specifying the kind of medication in use.

2.2 Electroencephalography

Electroencephalography (EEG) is a non-invasive method for recording electrical activity in the brain. EEG signals are recorded using electrodes placed on the scalp so that they capture signals simultaneously from most cortical brain regions. The measured voltage fluctuations are caused by populations of pyramidal cells of the cortex as a response to thalamo-cortical and cortico-cortical brain connections. These voltages are small, of the order of magnitude of several microvolts, which means that the signal is somewhat prone to artefacts from other electricity-producing activity near the electrodes, such as muscle movements. [24]

The voltage at each electrode is recorded in comparison with a common reference, producing signals unique to the location of the electrode. These signals are in the format of waveform time series, which can be used in electrophysiological analysis by determining various features, such as frequency, phase and amplitude, from the data to quantify the raw signals. [25]

EEG is regarded as having a high temporal resolution, of the order of magnitude of milliseconds, which makes it one of the best methods for recording real-time neuronal activity of the brain. Conversely, the spatial resolution of EEG is usually mentioned as being poor. This is often stated as a downside of the method, as it makes localization of findings often too imprecise for matching the activity to the underlying structural brain areas. In clinical use, EEG is particularly useful for evaluating seizure activity and epilepsy. Other indications for its use include monitoring recovery from hypoxic states of the brain. [24][26][27]

EEG can be efficiently used in connectivity analysis, as correlations between signals from all pairs of electrodes can be measured with different methods to suit the underlying hypothesis that is being tested. Thereafter, the correlations are used to define connectivity matrices to describe brain networks. Properties of these networks can then be analysed using, for example, measures of graph theory. [28]

There are potential problems in using EEG recordings for connectivity analysis, which must be taken into account in choices of connectivity metrics in order to minimize false correlation and other artefacts. One of these is the common reference problem, which arises from the fact that the EEG signal at each electrode reflects the difference of potential at the electrode and at the reference electrode. This means that fluctuations at the reference electrode are reflected in all signals produced this way, potentially causing false correlation at a zero time lag between two actually

uncorrelated electrodes. Other problems include the volume conduction problem, which is caused by currents flowing in tissues resulting in activity from one source being picked up by multiple electrodes. [28]

2.2.1 Infant EEG

Compared to adult EEG, neonatal EEG has a better spatial resolution and non-redundant information can be collected even with more scalp electrodes, due to the higher conductivity of the neonatal skull [29].

There are some features of EEG that are characteristic for perinatal children, and also some features which change quickly along with the conceptional age of the child [25]. This makes EEG an interesting method for studying the brain development in the very early stages of life. These features include trace alternant activity and increasing continuity [25], and spontaneous activity transients (SATS), which include so called delta brushes [30][3].

Age-dependent features of neonatal EEG can be used in determining the conceptional age of the baby, and consequently in the assessment of whether the EEG of the baby is normal. The interpretation of these features is traditionally based on the assumption that the immature brain develops at the same rate both in utero and after delivery [25]. This may not always be true, because it has been shown that some connectional features in preterm neonates differ from full-term neonates of corresponding age [31].

One of the features of normal EEG in newborn infants is interhemispheric synchrony (IHS). IHS is defined as temporal co-incidence of activity across brain hemispheres, which is usually encountered during trace alternant activity. [32]

EEG is clinically used for monitoring neurodevelopmental status of newborns and it is considered as a valuable tool for trying to minimize injury to the brain in neonatal intensive care [11][3]

2.2.2 Sleep EEG

The EEG data used in studying functional connectivity of the neonatal brain is usually recorded during sleep. This is due to the fact that the inevitable movement of the babies in the awake state would cause excessive artefacts in the recordings and lower the quality of the data beyond the needs of connectivity analysis [33].

By conventional definition, two different behavioral states, active sleep (AS), also called REM sleep, and quiet sleep (QS), also called non-REM sleep, can be distinguished in neonatal sleep EEG. Until conceptional age of circa 30 weeks, the EEG looks similar in both sleep states, whereafter the features of the sleep states have a differential development [26]. Active sleep constitutes up to 50 percent of sleep time in neonates, and its amount diminishes gradually by age, reaching 30 percent by the age of two and eventually 20 percent in adulthood [34].

The recognition of the sleep state usually has to be conducted visually based on EEG patterns, regularity of respirations, and the presence or absence of rapid eye movements (REMs) [35][26]. REMs occur during active sleep, but they are absent

during quiet sleep. EEG features of each sleep state vary greatly with the gestational age of the infant. [35]

In addition, functional connectivity has been shown to change significantly in neonatal test subjects between the two sleep states. [33].

2.3 Brain networks

Brain network science is a developing field of study, which incorporates observations from brain measurements into theoretical knowledge of complex networks to reveal the prevalence of network integration in the human brain [6]. Research has shown that both structural systems and functional systems in the brain have features of complex networks, which has led to many studies investigating brain networks in diverse experimental modalities [36].

While structural (anatomical) connections naturally occur between brain regions and neurons connected by white matter tracts, functional connections, consisting of correlated activity, may occur between anatomically unconnected brain regions [5]. Therefore, in the context of brain connectivity, it is important to make the distinction between structural connections and the statistical connections showing up in brain network analysis, because the observations in EEG connectivity analysis are not necessarily representative of the underlying physical connections. Nevertheless, the structural connections act as a foundation upon which the functional connections may develop, and should therefore be regarded as constraints on the overlying functional networks [4][6]. How the anatomical networks relate to the segregation and integration observed in statistically reconstructed functional networks is an interesting outstanding question in brain network science [6].

Graph models are often used as a tool to examine brain network organization, topology and complex dynamics [36]. In the early days, graph theoretical approach for understanding brain networks mostly dealt with binary networks constructed by the use of thresholding the data input [37]. With the development of more sophisticated technical and mathematical methods, the present research usually incorporates weighted networks as to more accurately reflect patterns of variable connections.

Using the graph theory approach, various organizational and topological properties have been found in the human brain [36][6]. Brain networks have often been characterized by small-worldness, similarly to many other complex systems, as in they generally have a high, non-random local connectivity, and short, near-random path lengths [4]. These are seen in network measurements, for example, as a high clustering coefficient and a high global efficiency [38][4]. Randomness of a network ensures fast spreading of information in a network, and regularity gives rise to coherent oscillations, which is why a brain as a small-world network takes advantage of the best of both features [39]. The small-worldness of brain networks has been interpreted to reflect two important features of the brain: highly specialized and localized processing of information, and cost-effective transfer of information from one area of the brain to another [40]. Additionally, modular organization is often revealed in the brain networks so that distinct clustering patterns and subnetworks

can be recognized [6].

2.3.1 Functional connectivity

By definition, functional connectivity means statistical dependence between time series of measured neurophysiological signals. Two spatially separate locations are regarded as functionally connected if they have synchronized dynamics, which can be used to study the inner workings of the brain. [4] EEG is a good tool for analysing the functional connectivity of the brain, because its high temporal resolution means that dependence can be studied in multiple time scales [41].

Functional connectivity of brain networks crucially affects the way the brain processes information, leading to the notion that simple activation studies previously conducted in brain research need to be replaced, or at least complemented, by more complex functional network studies [42].

Different research methods, such as fMRI and EEG, have been shown to express correlation in studying vigilance-dependent changes in dynamic functional connectivity networks [43].

Current research shows that both phase and amplitude relations can be used to define large-scale brain networks depending on the underlying hypothesis, as they capture different dynamics of connectional activity in the brain [28][44]. In this thesis, connectivity matrices based on both phase-phase correlation (PPC) and amplitude-amplitude correlation (AAC) are used for defining graphs to be analysed with graph theoretical metrics.

Graph theory metrics, such as global connectivity and network clustering in neonatal MRI have been shown to correlate with cognitive performance at the age of five [45]. As the time resolution of EEG measurements is better than MRI measurements, the network connectivity could theoretically be more accurately studied with EEG. By studying preterm neonates it has been shown that EEG can be used to determine network measures which can also be found to correlate with cognitive outcomes [31]. The need for accurate methods for predicting potential cognitive developmental problems has been recognized, because research has shown that not all early developmental problems are predictive of disabilities in the later stages of development [46]. Furthermore, as endogenous brain activity is a prerequisite for the development of the neonatal brain, finding relevant disruptions in the functional connectivity of the brain as early as possible is a task of clinical importance [47].

2.3.2 Development of brain networks

Functional connectivity is known to develop rapidly with age in the early stages of life. In the neonatal period, a neurodevelopmental change seems to occur from experience-independent development, which is essential in utero, to experience-dependent development, which is useful when there exists environmental sensory input. [33]

Early development of brain connectivity networks is thought to be driven by endogenous signals called spontaneous activity transients (SATs) [3]. SATs provide the brain with the possibility for innate activity-dependent wiring before there is

brain activity driven by sensory experiences [48]. In the proximity of full-term age, genetic factors and sensory factors are mutually responsible for the development of the brain [49][50]. Soon after the birth, SATs can no longer be seen in a healthy infant brain and their presence can indicate underlying pathology [3]. These processes can be seen as an indication of a gradual transition from experience-independent development essential in utero to experience-dependent development useful with the environmental sensory input [33].

The different functional connectivity measures have been shown to work as indicators of brain development. For example, as higher frequencies in the neonatal EEG are mostly incorporated into SAT events, amplitude fluctuations of higher frequencies are thought to indirectly indicate occurrence of SATs [51]. AAC coupling declines from preterm infants towards term age, and can also be seen as preterm infants having overall stronger AAC networks than healthy controls [33][31]. This decline reflects the disappearance of SAT events and has been suggested to be particularly fast in the weeks following term age [33]. Also, changes in PPC coupling can be attributed to maturing of the anatomical neuronal connections, as higher frequency correlation increases with age in the neonatal brain [33].

Synchronized neural connectivity develops from birth until early adulthood. The age-related changes of patterns in synchronization of high-frequency oscillations have been shown to correlate with both cognitive scores and resting-state brain activity. Additionally, the activation of frontal regions of the brain has been shown to increase as subjects approach adulthood, which in combination with precise synchronization has been suggested to be a landmark of maturity. [18]

GABAergic interneurons and gap junctions have been suggested as being a cellular level explanation for what causes synchronization of oscillatory in the brain, and these have been shown to play an important role in early brain development [18]. Maturation of GABAergic brain circuits have also been shown to determine the onset of critical developmental periods early in life, with the insight that pharmacological targeting of these circuits can onset premature or delayed development [19].

In general, networks in the infant brain are thought to be more globally dispersed, before being organized into more localized, anatomically proximal subnetworks seen in the adult brain [6].

2.4 Aims

The present thesis aims to find indications that functional connectivity as recorded with EEG could be used to examine the effects of AEDs on the development of brain networks in infants. Similar methods have previously been used successfully to find effects of other conditions, such as preterm birth [31]. Specifically, network metrics that have previously been found to discover differences in development near term age are in the focus of this study.

In addition, the results are also compared to the neurodevelopmental scores collected later to assess whether the differences seen in the brain networks are robust enough to show up in developmental performance of the children later in life.

3 Materials and methods

In this section, the data and research methods are described. First, general information about the dataset used in this thesis, as well as information about the data collection and preprocessing is introduced. After this, there are descriptions about how the methods of analysis work.

3.1 Dataset

The data used in this thesis consists of adjacency matrices of the size 58x58, where 58 is the number of nodes in the graph representing brain network.

These adjacency matrices were derived from AS and QS EEG measurements from 56 neonates fetally exposed to anti-epileptic drugs and 67 controls. The mean conceptional age during the collection of EEG data was 42 weeks. Of the AED group, 19 were exposed to oxcarbazepine or carbamazepine, 5 were exposed to valproic acid, 16 were exposed to polytherapy (none of which received valproic acid) and 16 were exposed to monotherapy with other drugs. For a more accurate description of the test subjects, the reader may refer to [2], where the same test group was used.

The EEG data was filtered into frequency bands around 21 central frequencies between 0.5 Hz and 19.2 Hz.

The data was collected using 19 scalp electrodes placed using the international 10-20 standard. For information about the process of data collection, the reader may refer to http://www.babacenter.fi/methods/physiological-methods.

Using an infant head model and a cortical surface model for infant brain, the contribution of cortical current sources to each of the scalp electrodes can be estimated. Parcellation of these cortical sources was performed so that the eventual data consisted of 58 parcels representing brain regions all around the brain. These parcels became nodes for the adjacency matrices used in this thesis.

After reconstructing EEG signals for each of the 58 parcels, correlations between all signal pairs were computed. AAC was computed by mutually orthogonalizing the parcel signals, computing amplitude envelopes at each time point and calculating the Pearson correlation coefficient between these amplitude envelopes. Mutual orthogonalization has been reported to be effective at minimizing the potential non-independence of signals caused by volume conduction of currents [53][54].

For PPC, the squared weighted phase lag index (dWPLI) was computed. DWPLI is an index of phase-synchronization that minimizes sensitivity to noise and volume-conduction [55].

For a more detailed description of the aforementioned preprocessing steps, the reader may refer to [31], where the data underwent similar processing.

3.2 Network measures

Network connectivity in the dataset was analysed using several measures: mean, modularity, efficiency, and local and global clustering coefficients. Different measures characterize distinct features in the networks, such as the presence of subnetworks or

Frequency	Type
0.50	Delta
0.60	Delta
0.72	Delta
0.86	Delta
1.04	Delta
1.24	Delta
1.49	Delta
1.79	Delta
2.15	Delta
2.58	Delta
3.10	Delta
3.72	Delta
4.46	Theta
5.35	Theta
6.42	Theta
7.7	Alpha
9.24	Alpha
11.1	Alpha
13.3	Beta
16.0	Beta
19.2	Beta

Table 1: List of central frequencies of the frequency bands used in EEG data and their corresponding wave types according to the conventional classification [52].

the level of integration [42]. For calculating the measures, Matlab functions provided in The Brain Connectivity Toolbox [5] were used, with the exception of mean, which was calculated using the Matlab default function.

In the following notations, N is the set of all nodes in the network, whereas n is the number of nodes. *(i,j)* is a link between the ith and the jth node in the graph, and it is associated with a connection weight w_{ij}, which is the correlation between the two nodes.

3.2.1 Mean

The mean connectivity, M, is simply calculated as the average of all weights in the network,

$$M = \frac{1}{n} \sum_{i,j \in N} w_{ij}. \tag{1}$$

The mean connection strength is used to indicate whether the overall connectivity in the networks is sparse or weak, or dense or strong.

3.2.2 Efficiency

Global efficiency is a measure for quantifying functional integration in a network, and it is defined as the inverse of average shortest path length in a network. It is related to another network measure called characteristic path length, with the difference that global efficiency can be also used for disconnected networks, as the inverse of an infinite path length of two separated network components produces an efficiency of zero. Global efficiency is also more affected by short paths than its counterpart, which is why it is usually regarded as the superior measure for functional integration in brain networks. [5]

Global efficiency, E, is calculated as

$$E = \frac{1}{n}\sum_{i\in N} E_i = \frac{1}{n}\sum_{i\in N}\frac{\sum_{j\in N, j\neq i} d_{ij}^w}{n-1}, \tag{2}$$

where d_{ij}^w is the shortest weighted path length between nodes i and j, and E_i is the efficiency of node i.

3.2.3 Clustering coefficient

Clustering coefficient is a measure for quantifying functional segregation in a network. The clustering coefficient of a node is equivalent to how much the neighbours of the node are also connected to each other, comprising triangles. The mean clustering coefficient is used for measuring global prevalence of clustered connectivity in a network as a whole. [5][4]

The clustering coefficient of a node i, Cl_i is calculated as described by Onnela and colleagues in [56]:

$$Cl_i = \frac{2t_i^w}{k_i(k_i-1)}, \tag{3}$$

where k_i is the degree of node i, and t_i^w is the weighted geometric mean of triangles around i. The network-wide, global clustering coefficient, Cl, is consequently calculated as

$$Cl = \frac{1}{n}\sum_{i\in N} Cl_i. \tag{4}$$

Both nodewise and averaged clustering coefficients are presented for the networks in this thesis as measurements of local and global clustering, respectively.

3.2.4 Modularity

Modularity is a summary statistic for quantifying the degree to which a network can be subdivided into separate, non-overlapping components. A higher modularity value indicates a stronger division into distinct network communities [6]. Like the clustering coefficient, it is classified as a measure of functional segregation in a network by Rubinov and Sporns in [5]. However, modularity measurements can also be classified separately from segregation measurements due to the difference in how they deal with within- and between-community connectivity structures in the network [6].

Unlike the other measures used in this thesis, modularity is not calculated exactly but instead estimated using an optimization algorithm due to the computational demand of the task. The Matlab function used in this thesis uses an algorithm described by Newman in [57] for optimizing the subnetwork placement for each individual node. It evaluates the division into subnetworks by returning a Q value that indicates how successful the division is: a value of 0 means the division is totally random, and a value of 1 means a perfect division [58].

The Q value for modularity returned by the function is calculated as described by Newman in [58]:

$$Q = \frac{1}{l_w} \sum_{i,j \in N} [w_{ij} - \frac{k_i^w k_j^w}{l^w}] \delta_{m_i,m_j}, \tag{5}$$

where m_i is module containing node$i$, and $\delta_{m_i,m_j} = 1$ if $m_i = m_j$, and 0 otherwise (if m_i and m_j don't belong to the same module).

3.3 Rank sum

Comparisons between groups were done using the Wilcoxon rank sum test [59]. This method pools all observations together, ranks them based on their position in the ascending ordering of all observation values, and determines whether the sum of ranks of values from both groups are similar enough to be from the same population. This was computed using the default Matlab function *ranksum*.

3.4 Post-hoc correction

When multiple statistical tests are conducted, the p-values need to be adjusted accordingly to account for positive findings that occur purely by chance. Multiple comparisons were corrected in the calculations of node-wise clustering coefficients using a method described by Palva and colleagues in [60]. In this method, the expected number of false positives is calculated according to the number of performed tests, after which that number of initially significant observations is removed in the order of descending p-value.

3.5 Network-based statistic

To complement the aforementioned network measures, the network-based statistic (NBS) method introduced by Zalesky and colleagues (2010) was applied to the connectivity matrices. NBS is a method developed for finding differences between brain networks and its purpose is to control for a large number of multiple comparisons while still remaining powerful, especially in networks with a low contrast-to-noise ratio. This is achieved by searching for connected components in the network and testing the null hypothesis on a component-by-component basis, as opposed to simply applying a link-wise correction based on each p-value as a separate occasion. [61]

In this method, a t-statistic threshold is selected to find a subset of edges which present the most difference in the pairwise association between the two groups. The

selection of this threshold is described as arbitrary. Using a breadth-first search, connected components among these suprathreshold edges are identified, and their size, k, is stored. Permutation testing is then used by randomizing the test subject group allocations for M (5000 in this thesis) permutations and finding connected components for each random permutation. A corrected p-value is estimated to determine whether the connected component is significant by calculating the number of maximum component sizes which are larger than k, and normalizing by M. [61]

The NBS works under the assumption that the statistically different links are connected to each other, and therefore can only find a single component of interest in a network. It can also only be used to reject the null hypothesis for the component as a whole, whereas more traditional methods with a more robust FWE-control can declare individual connections significant. [61]

The arbitrary selection of the t-statistic threshold has been listed as a source for criticism for this method, but this can be countered by noting that control of the family-wise error rate is guaranteed irrespective of the threshold choice [62]. In this thesis, a t-statistic threshold of 2.1 was used for most of the analyses. This value was chosen as a result of preliminary point checking of some instances where different subnetworks were expected after the assessment of clustering coefficients.

3.6 Network visualization

Visualization of the brain networks is an important part of showing the results of statistical correlations in this thesis, because looking at only matrices of p-values will not convey information as efficiently as possible. Therefore, the networks have been mapped to two-dimensional and three-dimensional models of the brain in order to make it easier to comprehend the results. Adjacency matrices of various frequency bands and correlation modes have been regarded as graphs so that each value corresponds to the strength of an edge between two nodes. With this method, the graph can be visualized as a brain model where the nodes denote cortical parcels and the edges denote a functional connection between them. The visualizations have been done with a Matlab toolbox called BrainNet Viewer [63].

3.7 Neurodevelopmental outcomes

For assessment of neurodevelopmental outcomes, the test subjects were tested at the age of two using the third edition of Bayley Scales of Infant and Toddler Development (BSID). BSID is an instrument for measuring the functioning development and possible developmental delays in infants and toddlers [64].

In this thesis, the BSID scores assigned to the test subjects for their developmental status in cognition, language skills in terms of both receptive and expressive communication, and fine motor and gross motor skills were used. Pearson correlation coefficient was computed between the BSID scores and connection strengths in the subnetworks found with the NBS analysis. For Pearson correlation, the Matlab function '*corr*' was used.

4 Results

This section explains what was found in the analysis of the connectivity matrix data.

4.1 AAC matrices

4.1.1 Mean connectivity

Firstly, the means of the correlation values that comprise the AAC networks were calculated to find out the overall strengths of the networks. No significant differences were found between the two research groups, AED and control, in the active sleep mode.

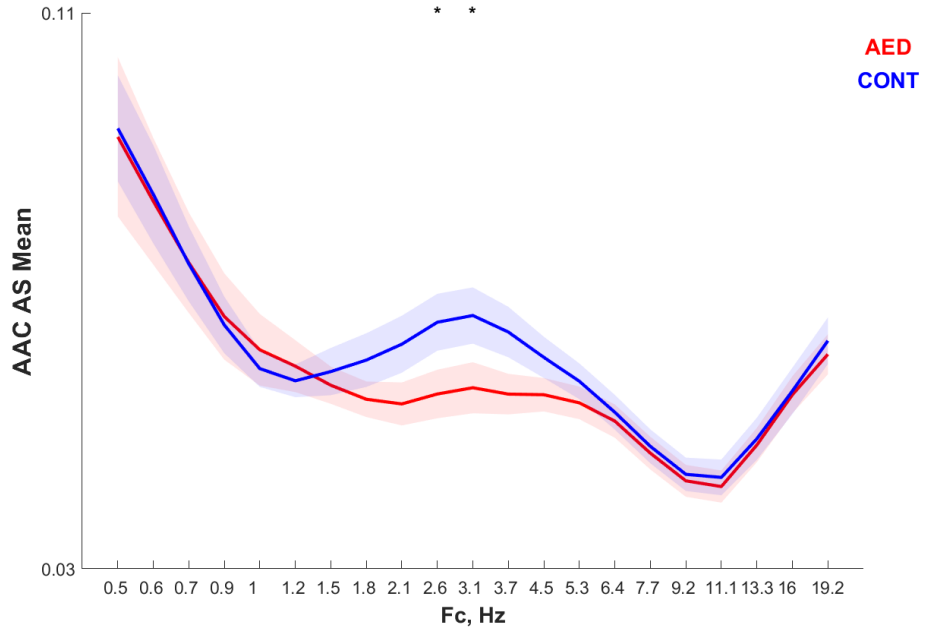

Figure 1: The mean connectivity of the amplitude-amplitude correlation connectivity matrices in active sleep mode. The small asterisks above frequency bands 2.6 and 3.1 Hz denote p-values less than 0.1.

In the quiet sleep mode, the means of the correlation values for the lower frequency bands, 0.5-0.6 Hz, were found to be higher in control group than in the AED group. In higher frequency bands, 13.3-16 Hz, a reverse effect could be seen, as the means were significantly higher in the AED group than the control group.

4.1.2 Modularity

The modularity of the networks did not show significant differences between the two research groups in either of the sleep modes in AAC. Only one frequency band in quiet sleep, 0.6 Hz, produced a p-value indicating significant difference, but these

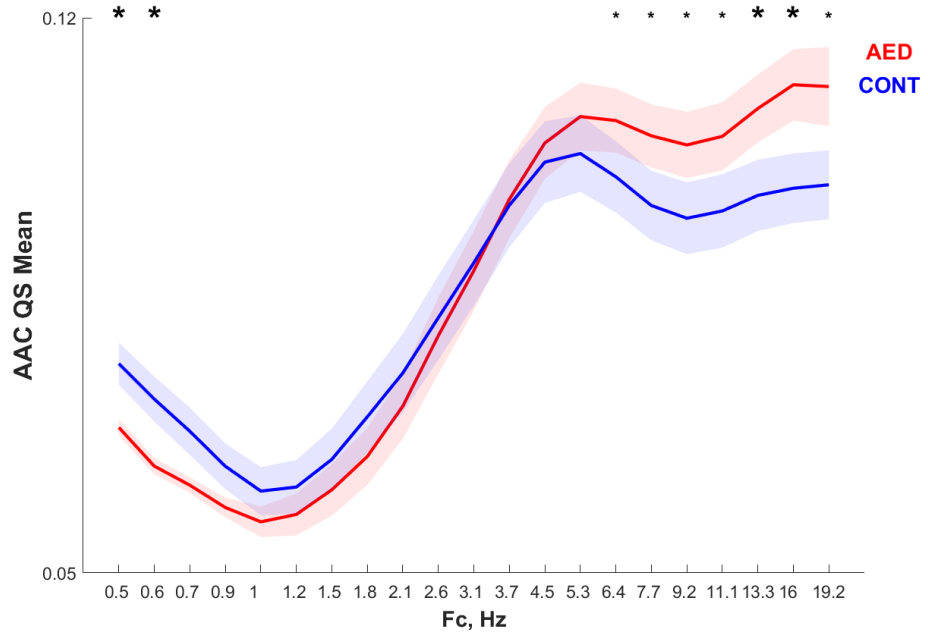

Figure 2: The mean connectivity of the amplitude-amplitude correlation connectivity matrices in quiet sleep mode. (Small asterisks: $p < 0.1$, larger asterisks: $p < 0.05$.)

tests were not corrected for multiple comparisons, which means that the result could be incidental.

4.1.3 Efficiency

The efficiency of the brain networks in AAC was calculated for both active and quiet sleep and the AED and control groups were compared to each other. In active sleep, a difference between the two groups was only seen in one frequency band, 2.6 Hz, where the efficiency of the control group was found to be higher than the control group. The higher and lower frequency areas did not show differences regarding the groups.

In quiet sleep, the middle frequencies did not produce differences between groups, but differences were found in both extremes of the examined frequency scale. In lower frequencies, 0.5-0.6 Hz, the control group produced a higher efficiency than the AED group. Conversely, in higher frequencies, 13.3-16 Hz, the efficiency of the networks in the AED group was found to be higher than the controls.

The efficiency of the networks seems to correlate with the previously mentioned overall strength of the networks. In active sleep, the efficiency of the networks was higher in lower frequencies with a decreasing trend towards the higher frequencies. In quiet sleep, the efficiency was found to be lower in the lower frequencies with an increasing trend towards the higher frequencies. This overall effect was present in both groups, AED and control, but it could be argued that the frequency dependent efficiency increase of the quiet sleep mode is more prominent in the AED group than

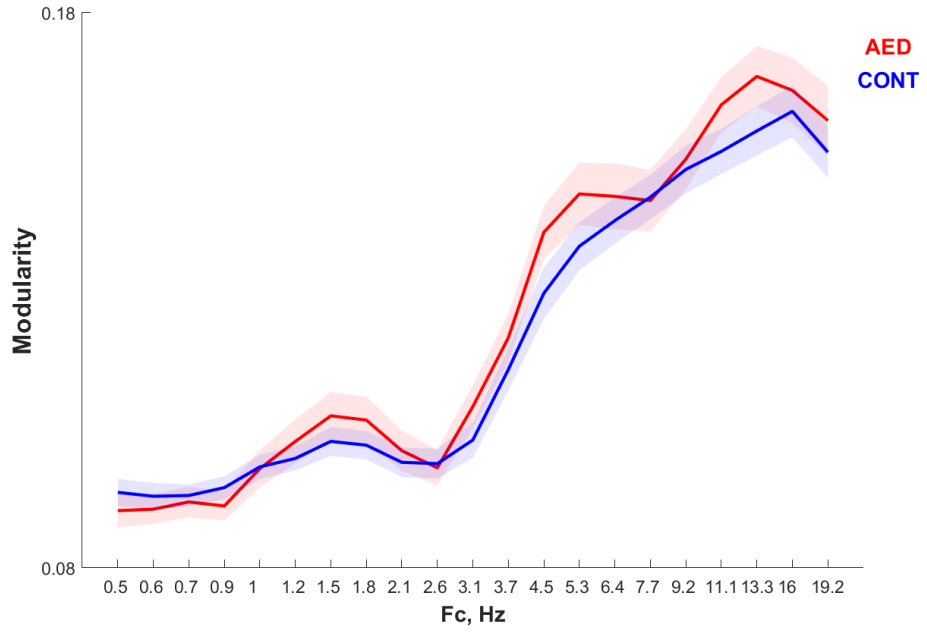

Figure 3: The modularity of the amplitude-amplitude correlation connectivity matrices in active sleep mode.

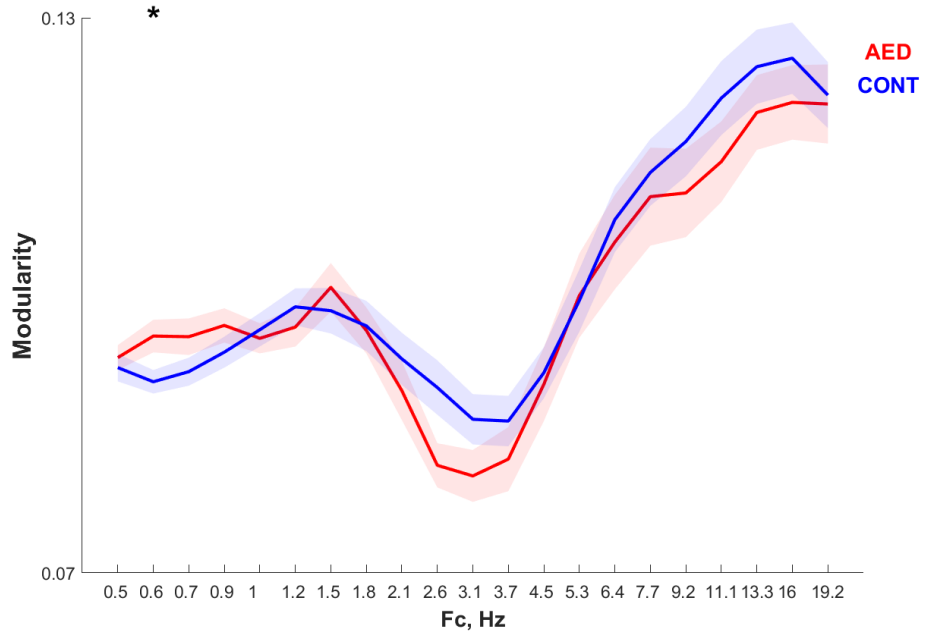

Figure 4: The modularity of the amplitude-amplitude correlation connectivity matrices in quiet sleep mode. The asterisk denotes $p < 0.05$.

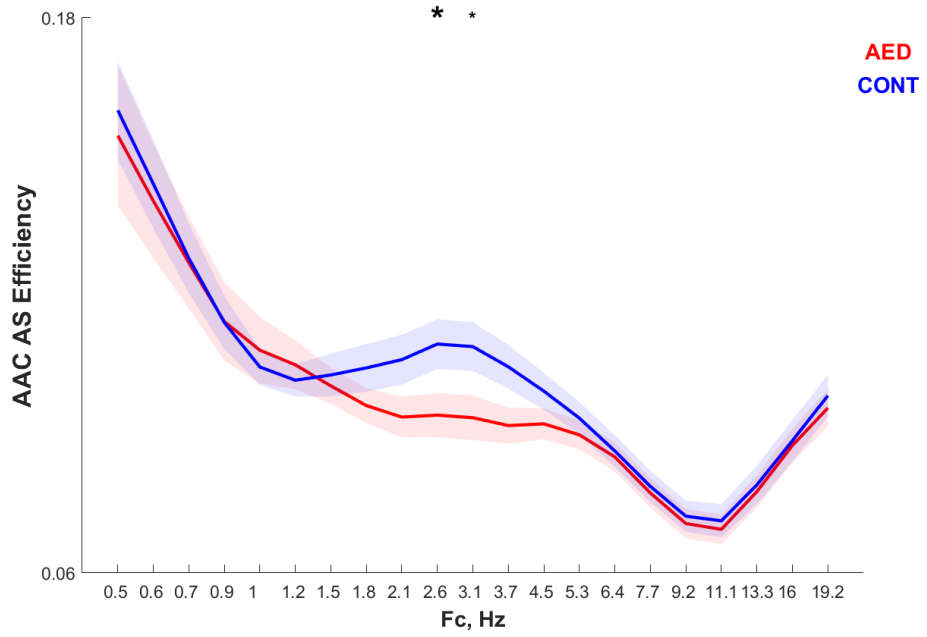

Figure 5: The efficiency of the amplitude-amplitude correlation connectivity matrices in active sleep mode. (Small asterisks: $p < 0.1$, larger asterisks: $p < 0.05$.)

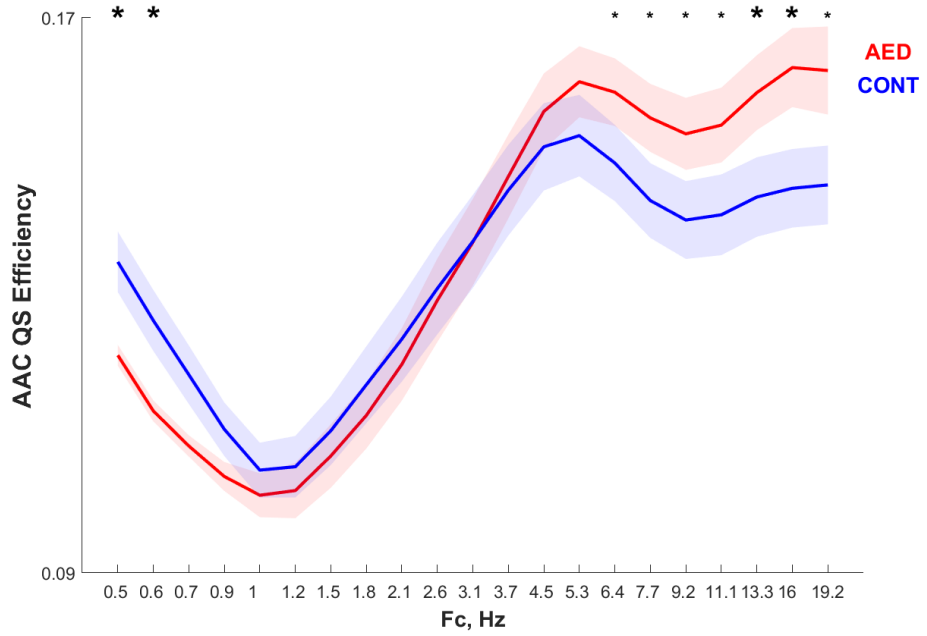

Figure 6: The efficiency of the amplitude-amplitude correlation connectivity matrices in quiet sleep mode. (Small asterisks: $p < 0.1$, larger asterisks: $p < 0.05$.)

in the control group.

4.1.4 Global clustering coefficient

Similarly to efficiency, the global clustering coefficient of the AAC networks was found to be different in some of the high (16 Hz) and low (0.5-0.6 Hz) frequency bands in the quiet sleep mode, with a similar trend of the AED group having lower clustering in the low frequencies and higher clustering in the high frequencies. Again, it can be noted that the clustering coefficient has more range in the AED group across the frequency bands than in the control group.

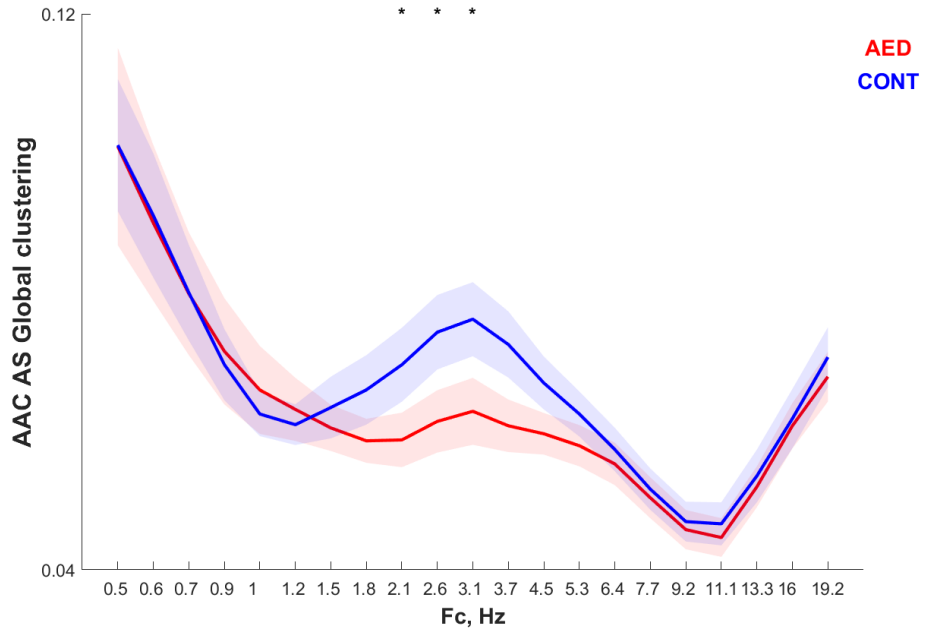

Figure 7: The mean clustering coefficient of the amplitude-amplitude correlation connectivity matrices in active sleep mode. (The small asterisks denote $p < 0.1$)

In the active sleep mode, the two research groups did not show significant differences between them in the global clustering coefficient.

4.1.5 Node-wise clustering coefficient

The clustering coefficient was calculated for each node of the AAC network in both active and quiet sleep, and the resulting coefficients were compared between AED and control groups. Correction for multiple comparisons was executed so that for all sets of 58 nodes representing a frequency band in each mode, the expected number of false positives was removed from the results. In the active sleep mode, it can be seen that in the frequency bands 2.1-3.7 Hz, there are multiple nodes in each network that have a higher clustering coefficient in the control group than in the

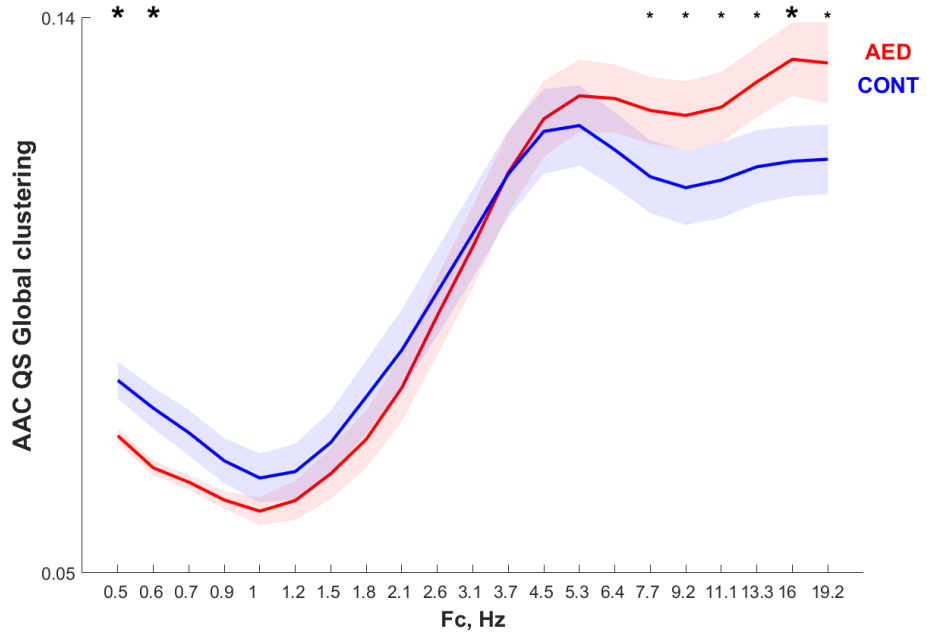

Figure 8: The mean clustering coefficient of the amplitude-amplitude correlation connectivity matrices in quiet sleep mode. (Small asterisks: $p < 0.1$, larger asterisks: $p < 0.05$.)

AED groups. These nodes don't show remarkable localization in the brain networks in visual inspection.

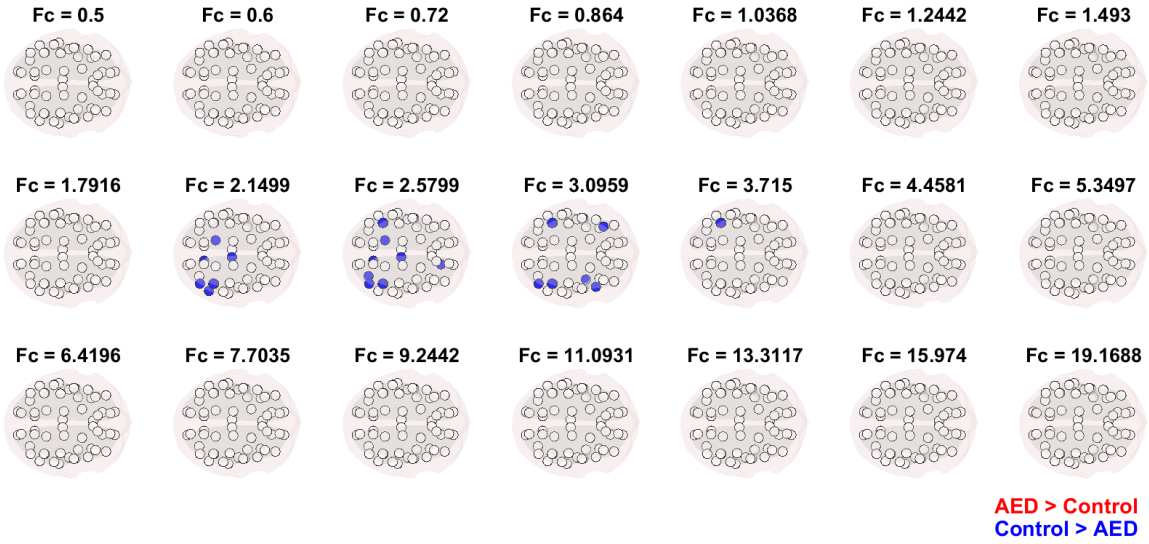

Figure 9: The node-wise clustering coefficients of the amplitude-amplitude correlation connectivity matrices in active sleep mode

In the quiet sleep mode, there are multiple nodal differences in the lower frequency bands, 0.5-0.6 Hz, where the nodes of the control group networks have a higher clustering coefficient than their counterparts in the AED group. In visual inspection, these nodes don't seem to be localized clearly to any specific region of the brain, but seem to be scattered across the brain network. In the higher frequency bands, 6.4-19.1 Hz, there are multiple nodes that have a significantly higher clustering coefficient in the AED group than in the control group. These nodes can be seen to have a weak localization emphasizing the frontal regions of the brain.

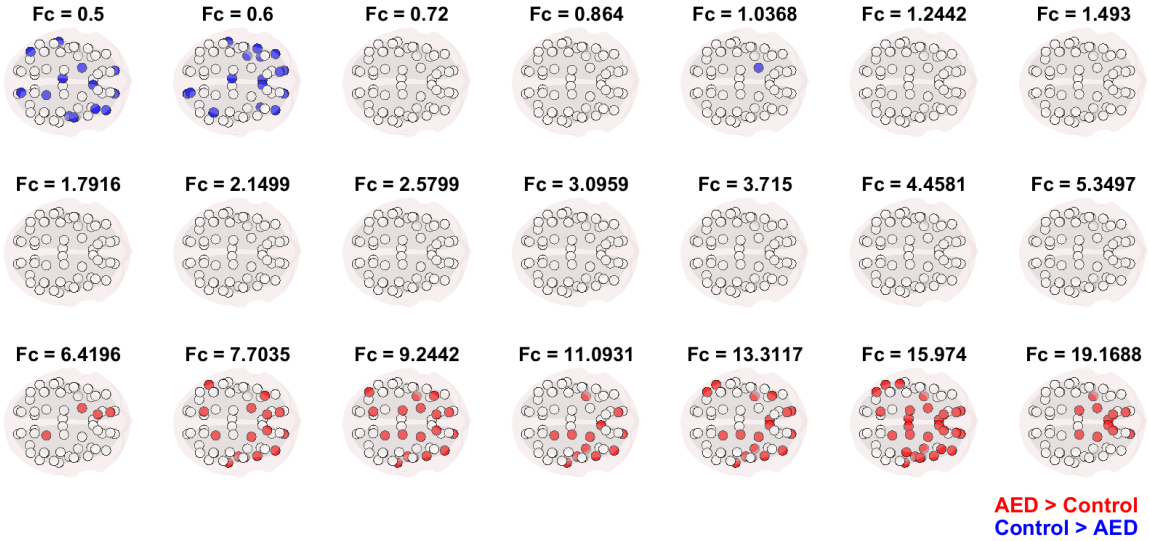

Figure 10: The node-wise clustering coefficients of the amplitude-amplitude correlation connectivity matrices in quiet sleep mode

4.2 PPC matrices

4.2.1 Mean connectivity

The mean correlation coefficient comprising the PPC networks researched in this thesis seems to decrease as the frequency increases. When comparing the AED and control groups, not many significant differences could be found. There was a significant difference in two isolated frequency bands, 1 Hz and 3.7 Hz ($p = 0.04$ and $p = 0.02$, respectively), in the active sleep mode. Additionally, a singular frequency band, 0.9 Hz ($p = 0.04$), could be found to show a difference between the two groups in the quiet sleep mode. In all of these frequency bands showing a difference, the mean correlation of the control group is higher than the AED group.

Also, even though the overall trend in both sleep states is that the mean correlation strength is negatively correlated with the frequency, there is still a visible difference in mean correlation between active and quiet sleep modes in both research groups.

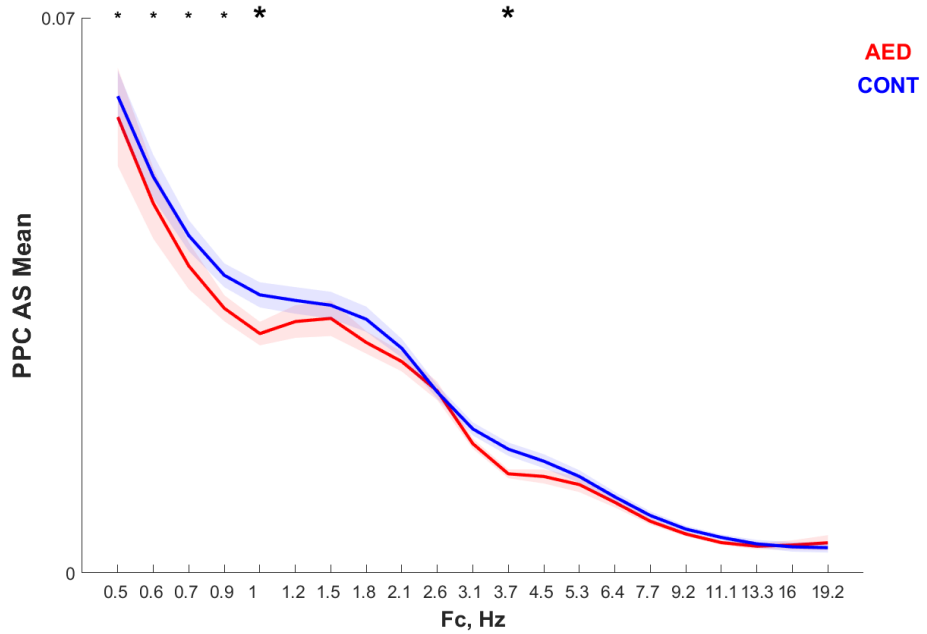

Figure 11: The mean connectivity of the phase-phase correlation connectivity matrices in active sleep mode. (Small asterisks: $p < 0.1$, larger asterisks: $p < 0.05$.)

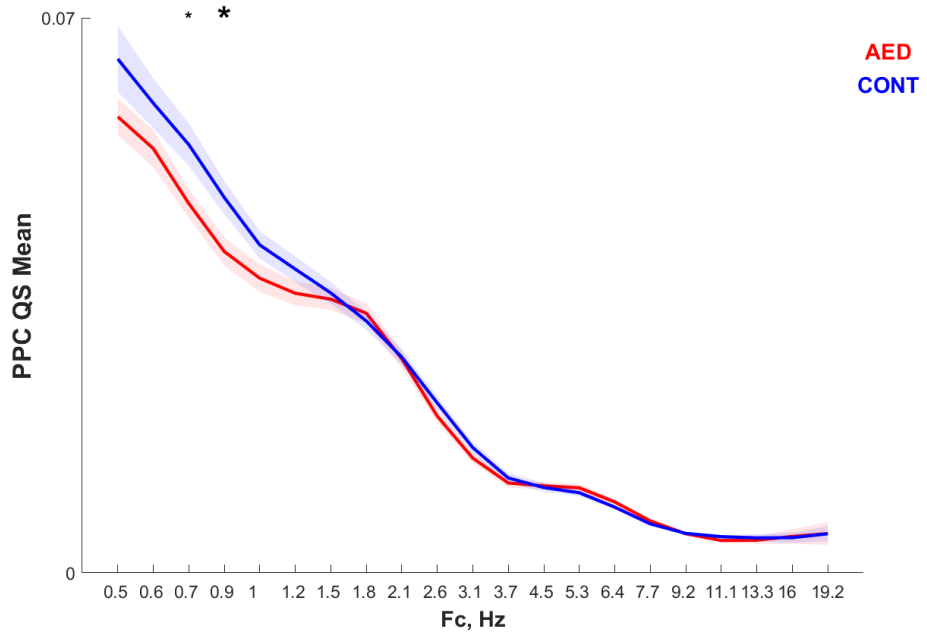

Figure 12: The mean connectivity of the phase-phase correlation connectivity matrices in quiet sleep mode. (Small asterisks: $p < 0.1$, larger asterisks: $p < 0.05$.)

4.2.2 Modularity

Whether the test subjects belonged to the AED or the control group could not be found to make a difference in the modularity of the PPC brain networks in either active or quiet sleep mode. Similarly to the AAC networks, modularity in PPC networks seems to be higher in the higher frequency bands than in the lower frequency bands.

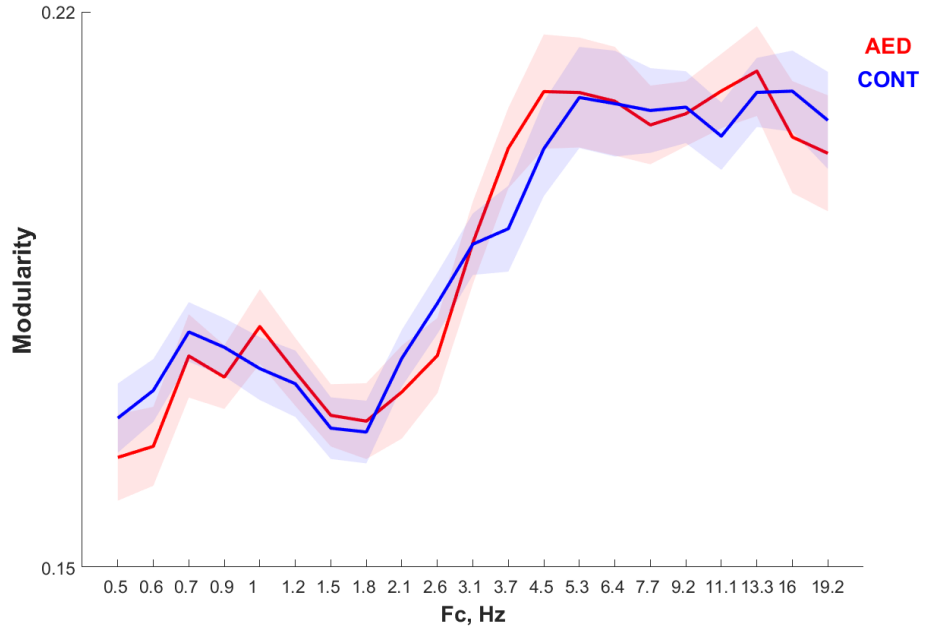

Figure 13: The modularity of the phase-phase correlation connectivity matrices in active sleep mode

4.2.3 Efficiency

The efficiency of the PPC networks was found to be higher in the control group than the AED group in some of the lower frequency bands in both active and quiet sleep. In the quiet sleep mode, the differences were presented in the frequencies 0.6-0.7 Hz and in the active sleep mode, difference could be seen in the frequency of 0.7 Hz. In addition to this, one frequency band in the middle of the researched frequency range, 3.7 Hz, showed a similar significant difference in efficiency. This singular finding coincides with the difference seen in the mean strength of the correlation network in the same frequency band.

4.2.4 Global clustering coefficient

The global clustering coefficient of the nodes in PPC networks was not significantly different between the research groups in either active or quiet sleep mode. Curiously,

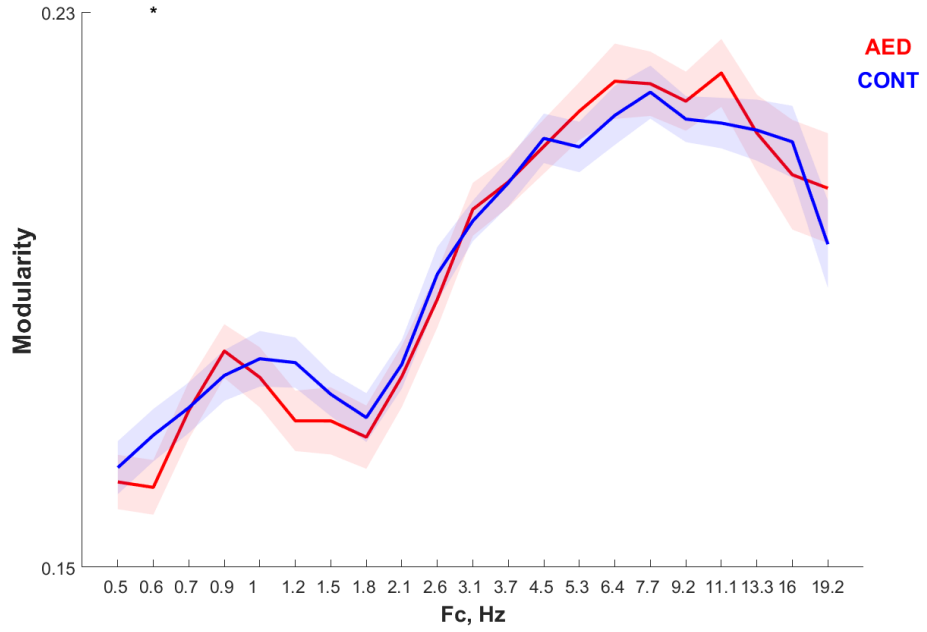

Figure 14: The modularity of the phase-phase correlation connectivity matrices in quiet sleep mode

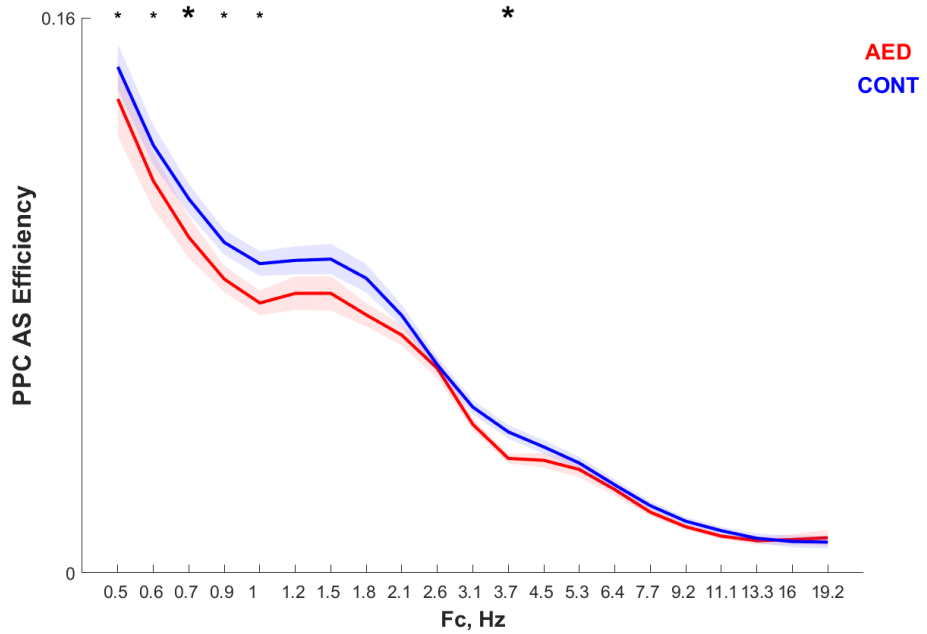

Figure 15: The efficiency of the phase-phase correlation connectivity matrices in active sleep mode. (Small asterisks: $p < 0.1$, larger asterisks: $p < 0.05$.)

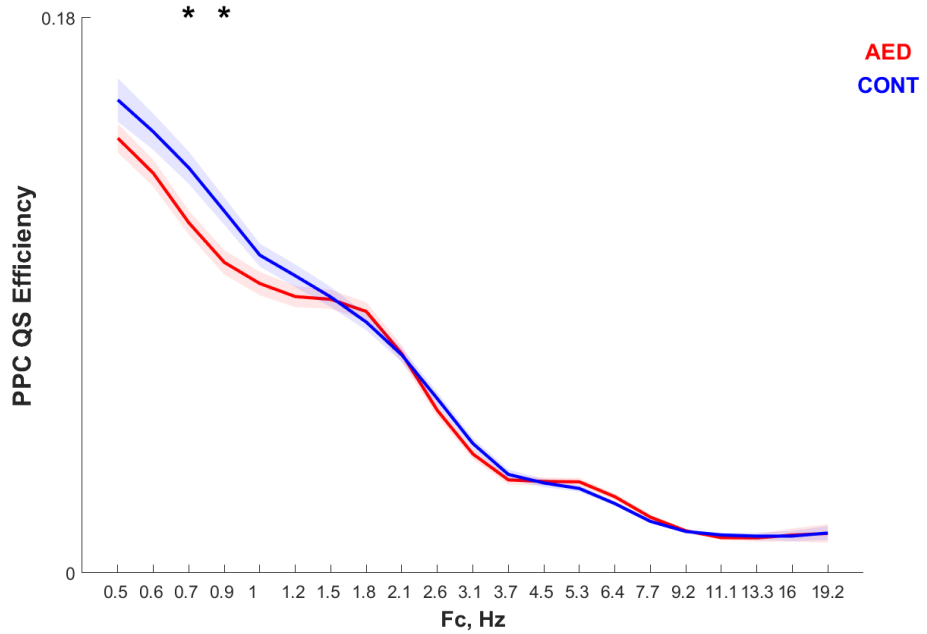

Figure 16: The efficiency of the phase-phase correlation connectivity matrices in quiet sleep mode. (The asterisks denote $p < 0.05$.)

there is one exception, which is the frequency band 3.7 Hz, where there was also a difference in efficiency and mean correlation in the same active sleep networks. In this frequency band, the control group showed a higher mean of the clustering coefficients.

However, there are multiple low frequency bands, 0.5-1 Hz, that almost reach the significant limit of $p<0.05$ in the active sleep mode networks. These frequency bands show a tendency of the control group having a higher clustering than the AED group, and it could be argued that multiple nearly significant consecutive findings as an aggregate would be significant. To prove this, further statistical analysis would need to be executed.

4.2.5 Node-wise clustering coefficient

There are differences between the two research groups in nodal clustering coefficients in multiple nodes across many of the frequency bands in the PPC networks. In the active sleep mode, frequency bands 0.5-1 Hz, 3.1-3.7 Hz and 11.1 Hz show more than three nodes with a significantly higher clustering in control group than in the AED group. Many of these frequency bands showed a global clustering reaching almost significant differences, which could mean that the local differences are not strong enough to show at a global level.

In the quiet sleep mode, the frequency bands 0.9-1.2 Hz, 3.1 Hz and 11.1-13.3 Hz show multiple nodes with a higher clustering coefficient in the control group than the AED group. There are also few singular nodes with a difference in frequency

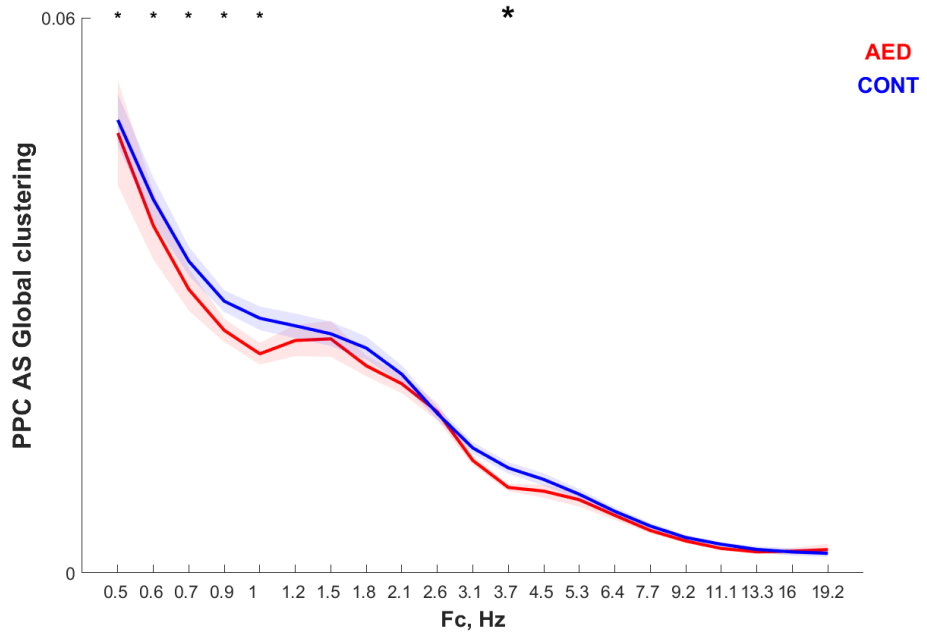

Figure 17: The mean clustering coefficient of the phase-phase correlation connectivity matrices in active sleep mode. (Small asterisks: $p < 0.1$, larger asterisks: $p < 0.05$.)

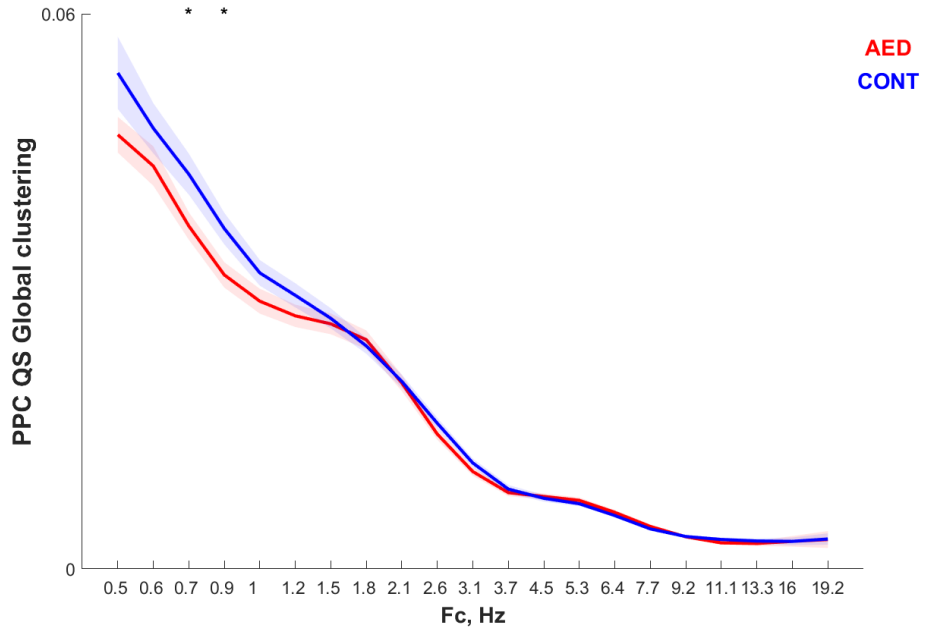

Figure 18: The mean clustering coefficient of the phase-phase correlation connectivity matrices in quiet sleep mode. (The small asterisks denote $p < 0.1$.)

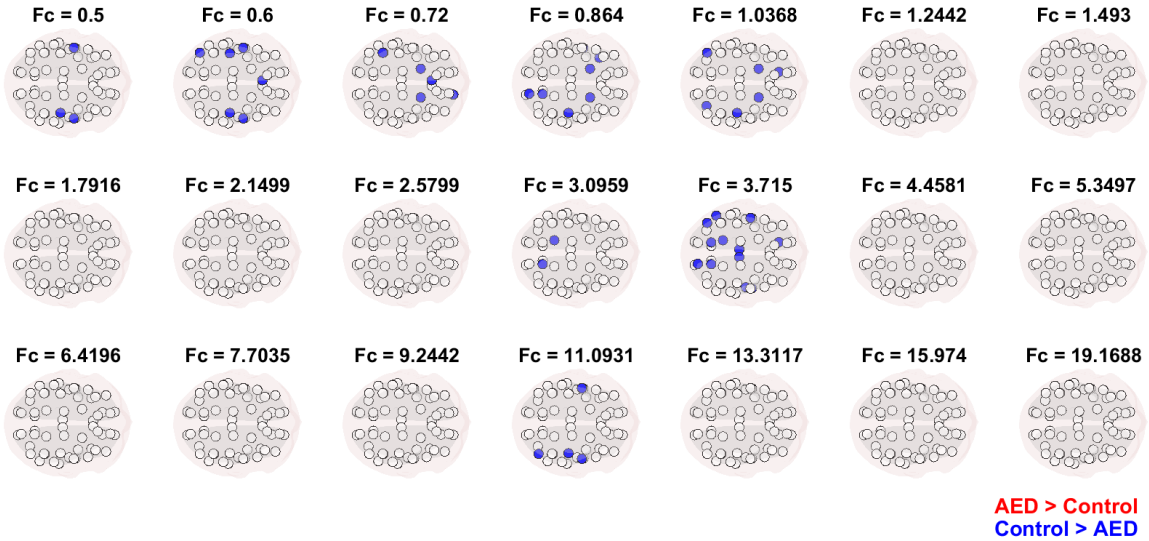

Figure 19: The node-wise clustering coefficient of the phase-phase correlation connectivity matrices in active sleep mode

bands 4.5 - 7.7 Hz, where the AED group has a higher clustering coefficient. Like in the analysis of node-wise clustering in AAC networks, the weakest three significant nodes were disregarded in terms of correction for multiple comparisons.

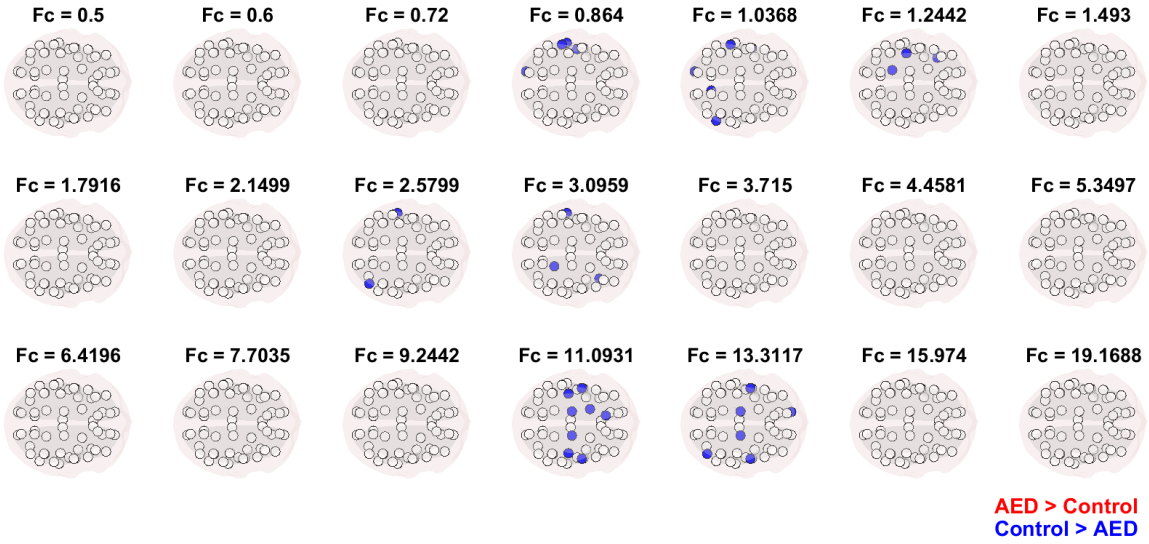

Figure 20: The node-wise clustering coefficient of the phase-phase correlation connectivity matrices in quiet sleep mode

4.3 NBS analysis

The NBS analysis produced results reflecting what was also found out in the local clustering analysis. In the frequencies where the networks presented with more concentrated areas of local clustering differences, subnetworks between nodes were generally found to be affected in the same direction.

The sizes of the largest subnetworks different between the AED group and the control group can be found by NBS can be seen in figures 21 and 22. In addition to the aforementioned findings, it can be noted that there are no subnetworks during AS in either AAC or PPC networks where the AED group would have a stronger connectivity. During AS, the control group had subnetworks with a stronger connectivity in multiple frequency bands, such as some coinciding findings in both AAC and PPC networks in the higher delta frequencies. During QS, the control group had subnetworks with a stronger connectivity in multiple delta frequencies.

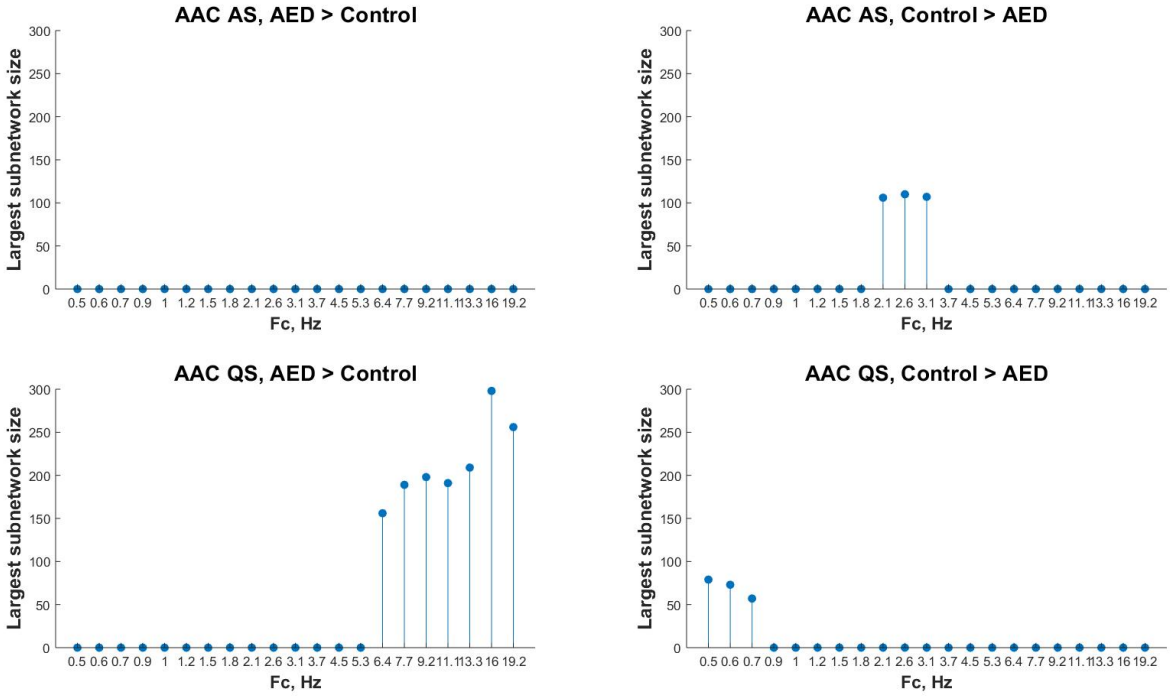

Figure 21: The sizes of significantly different subnetworks in AAC networks as produced by NBS

In AAC networks of QS mode, NBS analysis showed subnetworks with a higher connectivity in the AED subjects in multiple frequency bands. These frequencies were in the higher end of the tested frequency spectrum, the alpha and beta frequencies. These subnetworks were relatively dense and located mostly in the frontal areas of the brain, as seen in Figure 23. In the lower end of the frequency spectrum, subnetworks showing lower connectivity in the AED subjects could be found. These subnetworks fairly sparse and didn't show clear localization to any specific brain

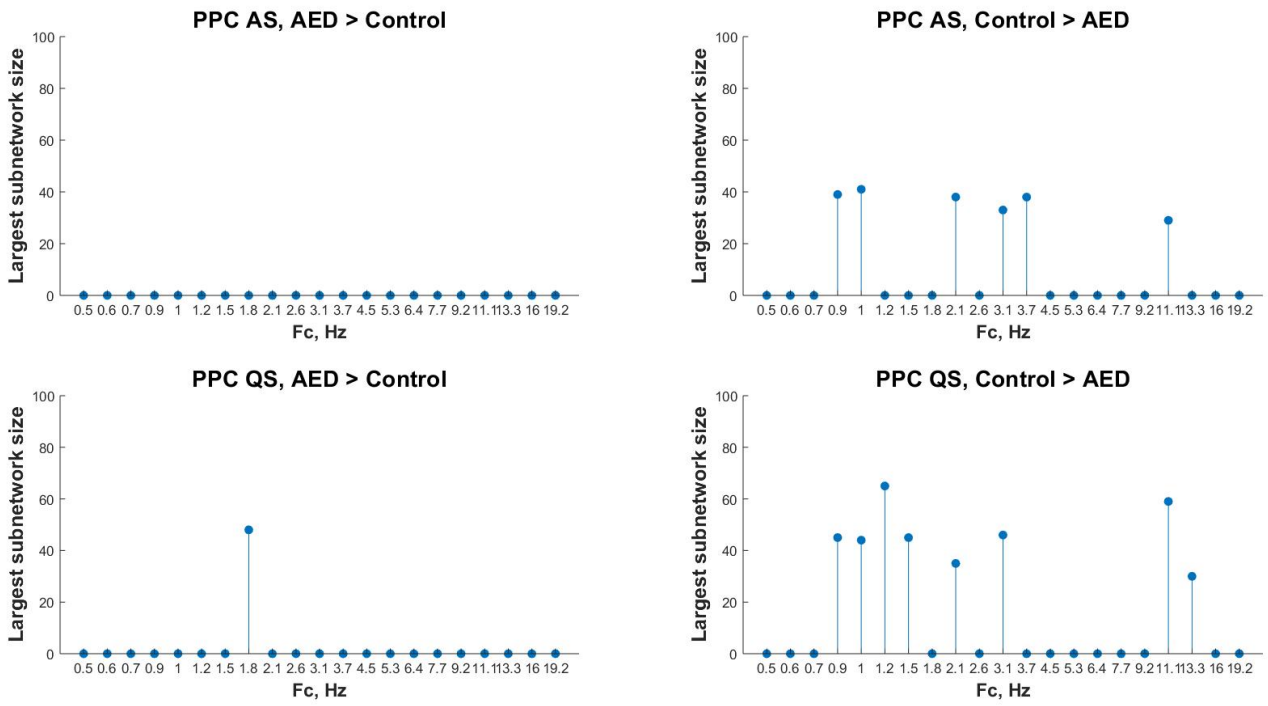

Figure 22: The sizes of significantly different subnetworks in PPC networks as produced by NBS

region, which is shown in Figure 24. These findings are well in line with the local clustering coefficients, which showed similar results in their part.

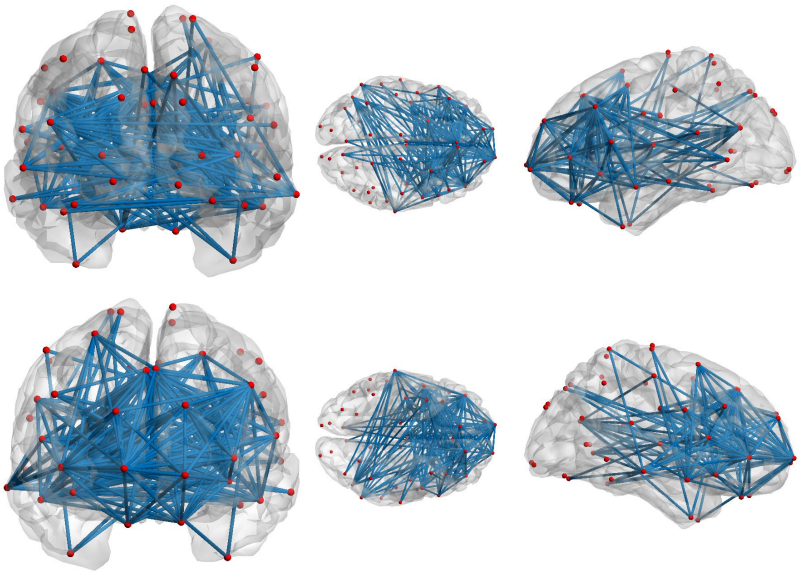

Figure 23: An example of a subnetwork of quiet sleep AAC connectivity (f=9.24Hz) that showed higher connectivity in the AED group than the control group in NBS analysis. In this picture, the top left shows the brain from behind, top center from the top, top right from the left, bottom left from the front, bottom center from the bottom and bottom right from the right.

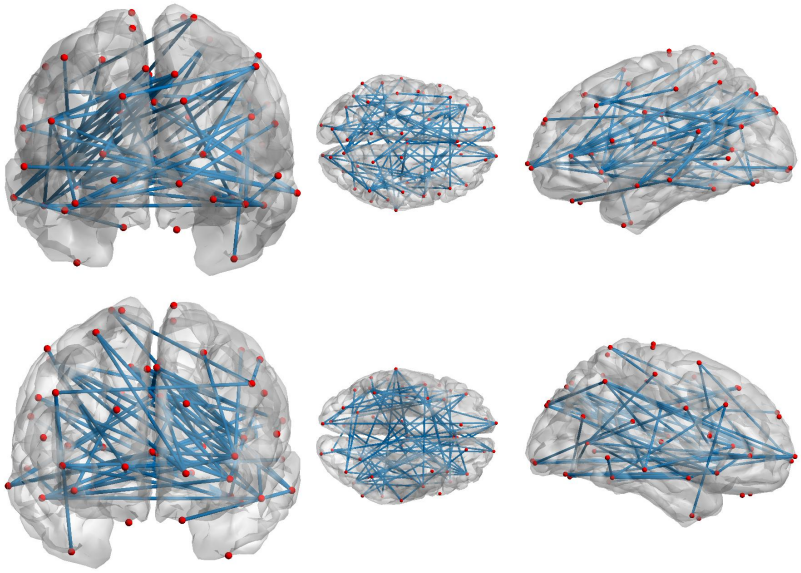

Figure 24: An example of a subnetwork of quiet sleep AAC connectivity (f=0.6Hz) that showed higher connectivity in the control group than the AED group in NBS analysis.

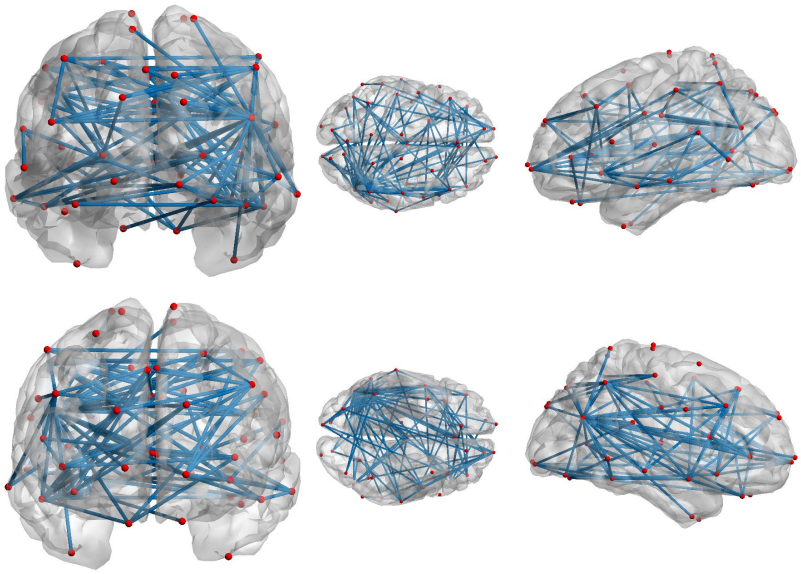

Figure 25: An example of a subnetwork of active sleep AAC connectivity (f=2.6Hz) that showed higher connectivity in the control group than the AED group in NBS analysis.

In AAC networks of AS mode, there were some significant differences in the higher end of delta frequencies, with the control group showing higher connectivity in some subnetworks. These subnetworks span all over the brain, with some focus in the right posterior area, which is seen in the Figure 25.

In PPC networks of QS mode, some subnetworks could be found as having stronger connectivity in the control group than the AED group. These networks covered many areas of the brain, but some localization could be seen to the left and posterior brain regions, as shown in Figure 26.

In PPC networks of active sleep mode, NBS analysis found subnetworks with significantly diminished connectivity in AED subjects. Interestingly, the subnetworks contained many interhemispheric connections near the center of the brain, as shown in figure 27. This finding complements the local clustering coefficient findings, which indicated lower clustering in some of the parietal and temporal nodes, but did not yet show the direction of the connections for which the correlation is lower.

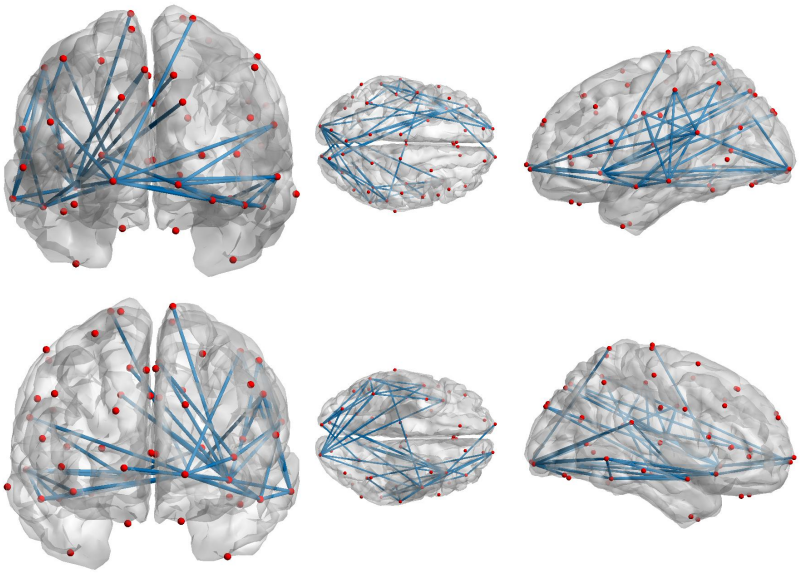

Figure 26: An example of a subnetwork of quiet sleep PPC connectivity (f=1.0Hz)that showed higher connectivity in the control group than the AED group in NBS analysis.

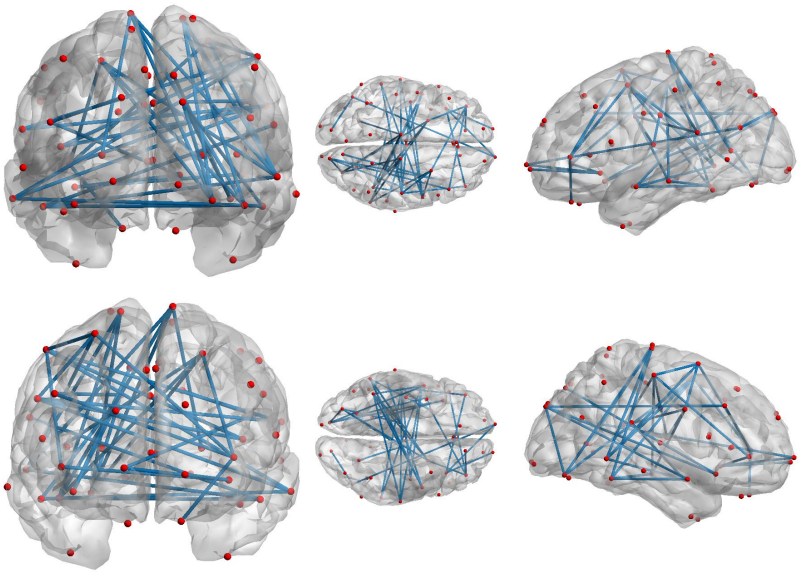

Figure 27: An example of a subnetwork of active sleep PPC connectivity (f=0.86Hz) that showed higher connectivity in the control group than the AED group in NBS analysis.

4.4 Neurological scores

Lastly, correlation between neurological BSID scores and the subnetworks indicated by the NBS analysis was calculated to find if the mean connection strengths in the subnetworks coincide with neurodevelopmental progress in the first years of life.

No correlation was found between the BSID scores and the subnetwork connection strengths in most cases. Only in PPC networks of AS, the subnetworks found in frequencies 3.1-3.7 Hz indicated correlation with the neurological scores ($p < 0.05$), namely in language expression.

	COG	LP	LE	FM	GM
3.1 Hz	0.6334	0.6575	0.0044	0.2256	0.9954
3.7 Hz	0.4709	0.7592	0.0292	0.6355	0.6047

Table 2: The neurological scores showing differences in language expression between control group and AED group in the connection strengths of the subnetworks found in NBS analysis in PPC of AS. *(COG = Cognition, LP = Language processing, LE = Language expression, FM = Fine motor, GM = Gross motor)*

5 Discussion

The results presented in this thesis show that there are differences between functional brain networks of infants exposed to prenatal AEDs compared to healthy controls. This is in line with the large body of data on neurodevelopmental problems these children are often encumbered with. It would be easy to say that AEDs should not be used during pregnancy, but AED use is in most cases a necessity for the well-being of the mother. Therefore, these kinds of results are most useful in learning to recognize the adverse effects of epilepsy medication early in life to be able to manage further complications.

5.1 General observations

In AAC networks of QS, the AED group had a higher clustering and stronger connectivity in the higher frequency bands, where as the clustering in the control group was higher in the lowest frequency bands. This could be seen as an overall shift of activity to higher frequencies in babies exposed to AEDs, but has in fact probably more to do with different developmental stages and their features. It could be speculated that the prominency of higher frequencies in the AED group is related to persistency of SATs for longer than expected, as the SATs encompass a lot of high frequency activity [25]. Higher delta wave activity in the control group could similarly be associated with better increasing of overall continuity of slow wave sleep, which occurs around the age of the infants studied in this thesis [26].

Subnetworks that were stronger in the control group than the AED group were found in many frequency bands of PPC networks. This is line with the notion that PPC networks increase in connectivity with the development of neonates [33], suggesting that the AED group might be experiencing slower development.

The subnetworks found in NBS analysis to be different in AED infants than healthy controls did not mostly correlate with neurodevelopmental assessment in the age of two. This is an indication of how difficult the task of predicting outcomes from EEG measurements is, and that these NBS results were not promising as biomarkers for slower development. It might be worth testing the same thing with different t-statistics in NBS, as the widespread nature of the results found with lower t-statistic values might confound the effects that a smaller, more focal subnetwork might bring forward.

5.2 Strengths, limitations and future directions

In this study, all infants exposed to AEDs were in the same study group instead of separating by pharmacological agent. Different AEDs are conventionally regarded as having different effects on the brain, which means that the studied effects on the brain were potentially largely heterogenous. Therefore, effects of any one drug might be confounded by others and not be seen with the methods used. Despite this, there were significant results in many network metrics studied here, which suggests further robustness of the results and the research methods. While all kinds of AEDs

have been shown to cause some complications for children of mothers with epilepsy, as mentioned previously, most of the adverse effects of AEDs are most strongly associated with valproate. It would be interesting to study the effects of valproate separately, as the differences in connectivity might prove more clear without other drugs with less neurodevelopmental effects dampening the results. This is probably not possible, though, as valproate use during pregnancy is (happily) decreasing because of the widely known recent findings [65]. Also, further research should be done about the comparative safety of different AEDs, for which functional network analysis such as in the present thesis might be beneficial.

There has been some speculation about how reproducible and reliable the EEG measurements of infants are. It has been suggested that while global measurements are more consistent in retesting, local measurements might not be as reproducible [66]. The results of local measurements, like clustering coefficients, in the present thesis seem fairly robust and it would be interesting to evaluate how well the results would hold up in retesting.

Only a few metrics describing properties of the brain networks were chosen for this thesis, with the aim of assessing whether there is promise for more extensive research. There is a diverse selection of network measures that could be used to get a more accurate view on the network properties that indicated possible significant results, which could also be used to validate the significances. For example, it would be interesting to use other measures of network segregation to see if they yield better or worse results than the clustering coefficients shown here.

When trying to find differences between the AED group and healthy controls, most of the network measures only rarely surpassed the p-value indicating significancy ($p < 0.05$). However, there seemed to be overall trends of nearly significant differences ($p < 0.1$) between groups in these measurements suggesting that the differences might be real and the power of these tests just wasn't enough to pass the "magical" significancy limit. Other methods of determining significancy, which take such group effect into account, could be investigated and used to find out whether these trends are really significant.

In NBS analysis, altering the choice of the initial t-statistic threshold produced a large variation in the resulting subnetworks. In preliminary visual inspection of the results, a lower initial t-statistic finds more subnetworks, but they are immediately larger than those found with a higher t-statistic, and often widespread across the whole brain, which then again makes assessment of potential localization harder. It is, of course, the purpose of the method to find subnetworks of maximal size, but for further analysis it might be beneficial to find the "sweet spot" where subnetworks can be found but they still show localization more clearly.

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